Report No. IITRI-L6023-6
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration

IIT RESEARCH INSTITUTE

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May 15 to August 15, 1966

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I. INTRODUCTION

Simulated Martian environment experiments with Bacillus cereus spores were concluded after 56 days. The effects of the following barometric pressures and carbon dioxide concentrations were studied:

(1) Earth atmosphere at pressures of 10, 25, 40, and 98 mb, with diurnal temperature cycling (-65 to 30°C).

(2) Carbon dioxide concentrations and pressures of 37% at 40 mb, 67% at 25 mb, and 100% at 10 mb, with diurnal temperature cycling.

(3) Carbon dioxide concentrations of 37, 67, and 100% at a pressure of 98 mb, with diurnal temperature cycling.

B. cereus spore germination was inhibited by carbon dioxide concentrations of 37, 67, and 100% at all pressures. Pressures as low as 10 mb with Earth atmosphere were not

inhibitory to spore germination, but vegetative cell growth was less than that at 98 mb.

Similar experiments were initiated to determine the effects of the same barometric pressures and carbon dioxide concentrations on Staphylococcus aureus. Earth atmosphere at 40 mb pressure with a constant temperature of 35°C or diurnal temperature cycling did not inhibit the growth of this organism.

Soil ecology experiments on the growth response of B. cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), and S. aureus in an alkaline California desert soil with 99% relative humidity and a constant temperature of 35°C or diurnal temperature cycling indicated that:

(1) L. plantarum, P aeruginosa, and S. aureus do not survive even at cell populations of 10^5 cells/g of soil.

(2) B. cereus and PA 3679 spores survive at spore populations as low as 10^2 spores/g of soil with less than a one log die-off after 56 days.

II. EXPERIMENTAL PROCEDURES

Procedures for growing B. cereus, P. aeruginosa, PA 3679, and S. aureus cultures were described in Report No. IITRI-L6023-5. L. plantarum was grown in trypticase soy broth (BBL) for four days at 22°C and then harvested and washed in the usual manner with 0.025 M phosphate buffer at pH 7.0. All stock cell suspensions were stored at 4°C until used. Spore suspensions were heat-shocked just before use.

Bacterial counts are reported as averaged counts of two plates from each of two tubes, after 24 or 48 hr. incubation at 35°C.

III. RESULTS AND DISCUSSION

A. Simulated Martian Environment

All tubes in these studies contained 1 g of felsite/limonite soil, 1% organic medium, and 7 to 10% moisture. The tubes were flushed sever times with a particular gaseous atmosphere before pressure was established; the tubes were then sealed. An 8 and 20 hr. diurnal freeze cycle was used with each atmosphere pressure.

1. B. cereus

Appropriate controls in these experiments consisted of tubes inoculated with B. cereus spores and after flushing, sealed at 98 mb pressure of Earth atmosphere. One-half of the tubes were incubated at a constant 35°C and one-half

at diurnal temperature cycle with an 8 hr. freeze. The tubes were sampled at 7 and 28 days. In all experiments the spores germinated with subsequent vegetative growth.

Experiments on the effects of barometric pressure and carbon dioxide concentration on germination of B. cereus spores were concluded after 56 days (Figures 1 to 10). As stated in Report No. IITRI-L6023-5, Earth atmosphere at barometric pressures of 10, 25, and 40 mb did not completely inhibit the germination of spores, although the growth responses were less than that at 98 mb (Figures 1 to 4). Spore germination with vegetative growth, evidenced by increase in total count, occurred at 10, 40, and 98 mb and to a lesser extent at 25 mb in tubes with 8 hr. freeze diurnal cycle. B. cereus spores maintained at 98 mb pressure with 20 hr. diurnal freeze cycle germinated and grew by 56 days.

Carbon dioxide concentrations of 37, 67, and 100% at pressures of 40, 25, and 10 mb, respectively, inhibited B. cereus spore germination for as long as 56 days (Figures 5 to 7). A loss of viability did not accompany inhibition of spore germination.

Figures 8 to 10 show the effects of 37, 67, and 100% carbon dioxide concentrations at 98 mb pressure on germination of B. cereus spores. Germination of spores was inhibited, but viability was not impaired in these environments.

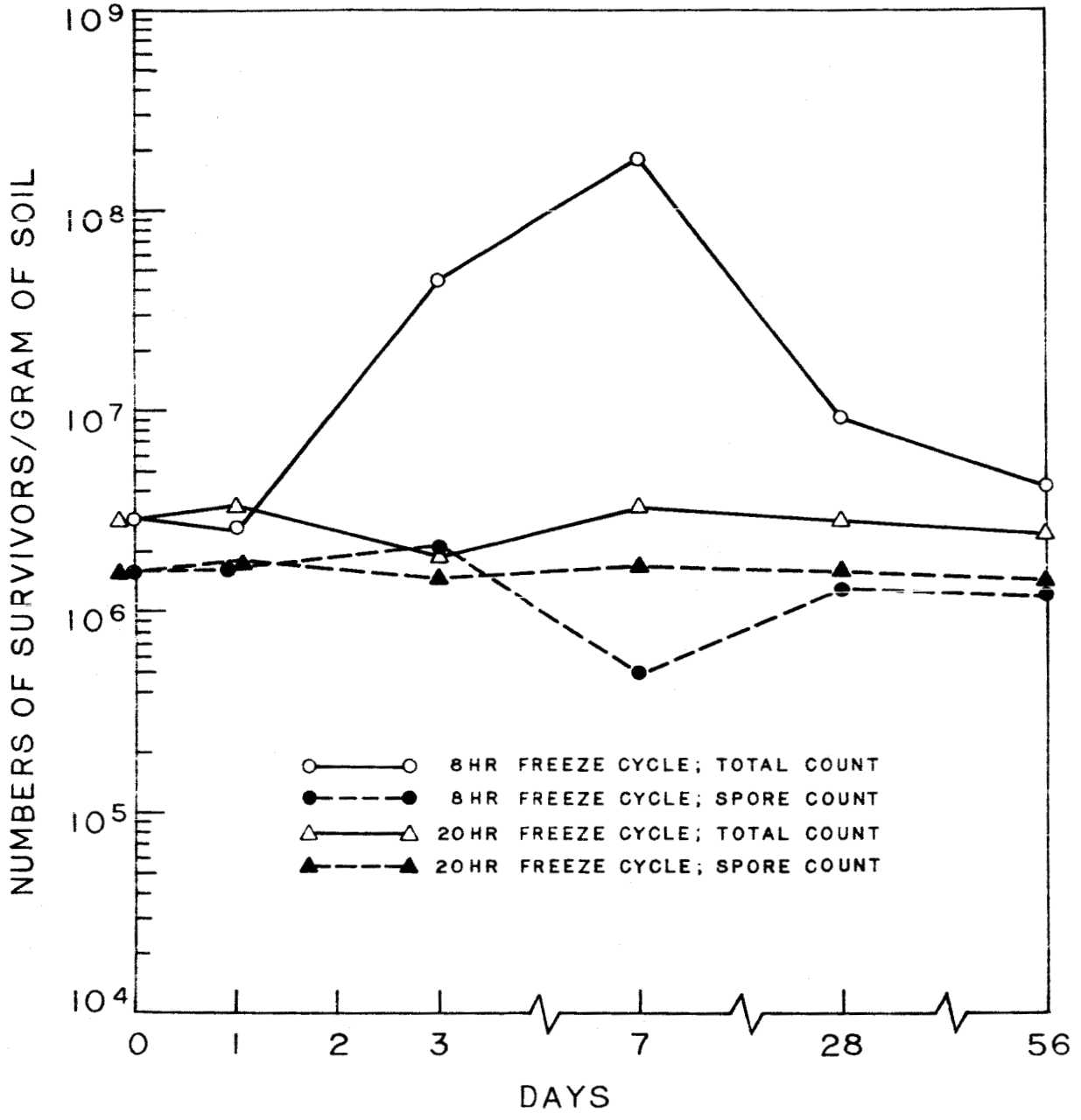

FIG. 1 THE EFFECT OF EARTH ATMOSPHERE AT 10mb PRESSURE ON BACILLUS CEREUS SPORES.

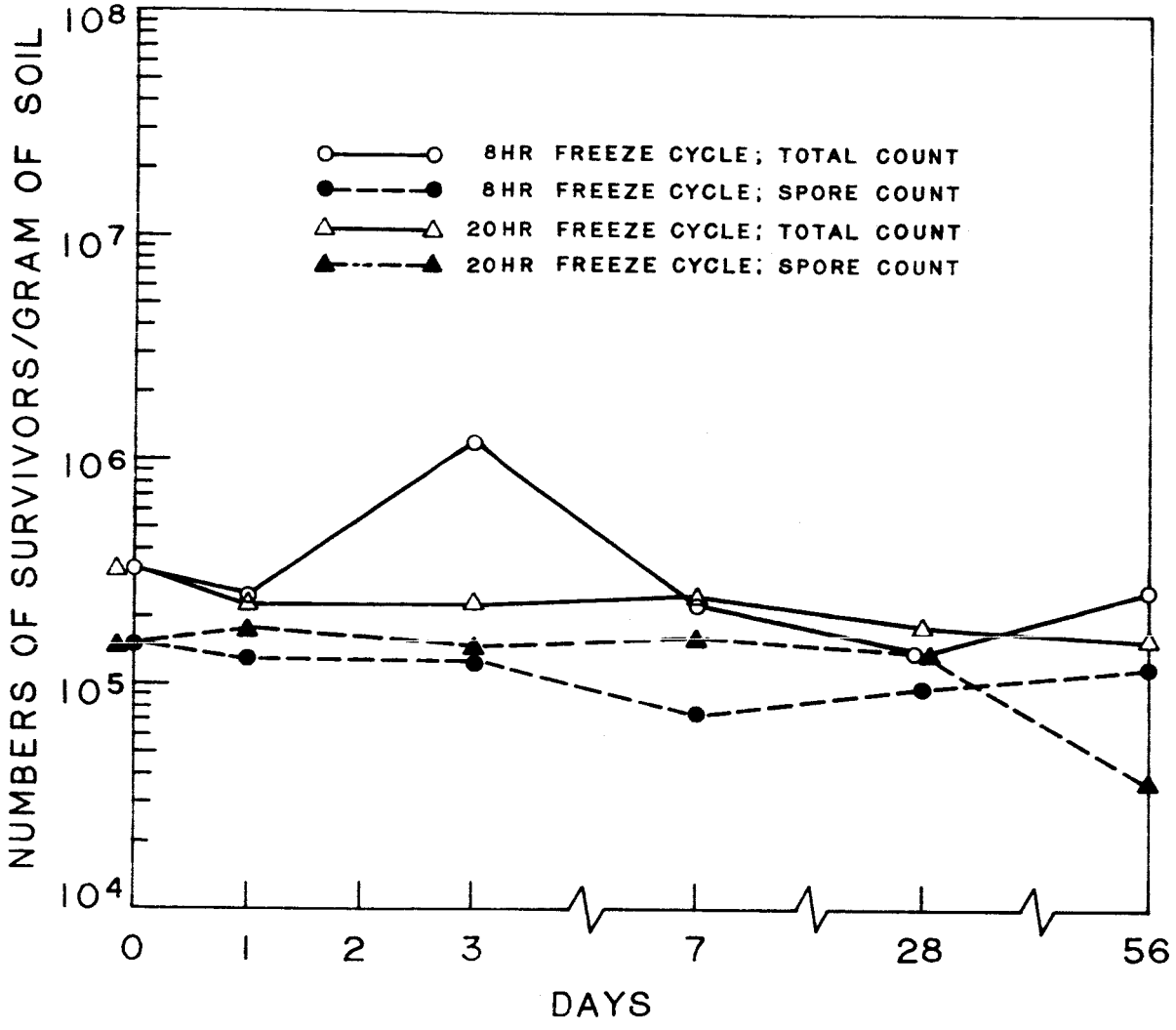

FIG. 2 THE EFFECT OF EARTH ATMOSPHERE AT 25mb PRESSURE ON BACILLUS CEREUS SPORES.

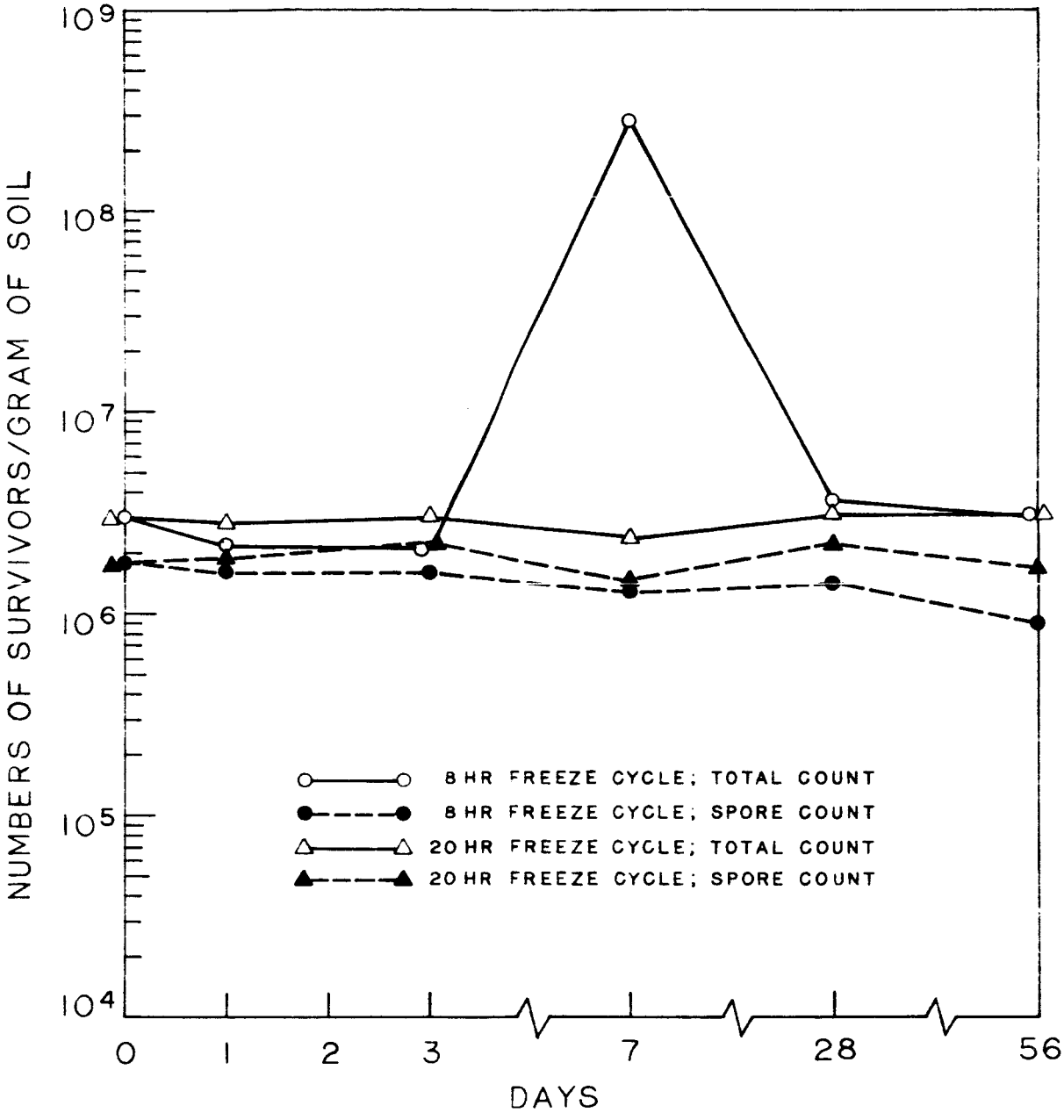

FIG. 3 THE EFFECT OF EARTH ATMOSPHERE AT 40mb PRESSURE ON BACILLUS CEREUS SPORES.

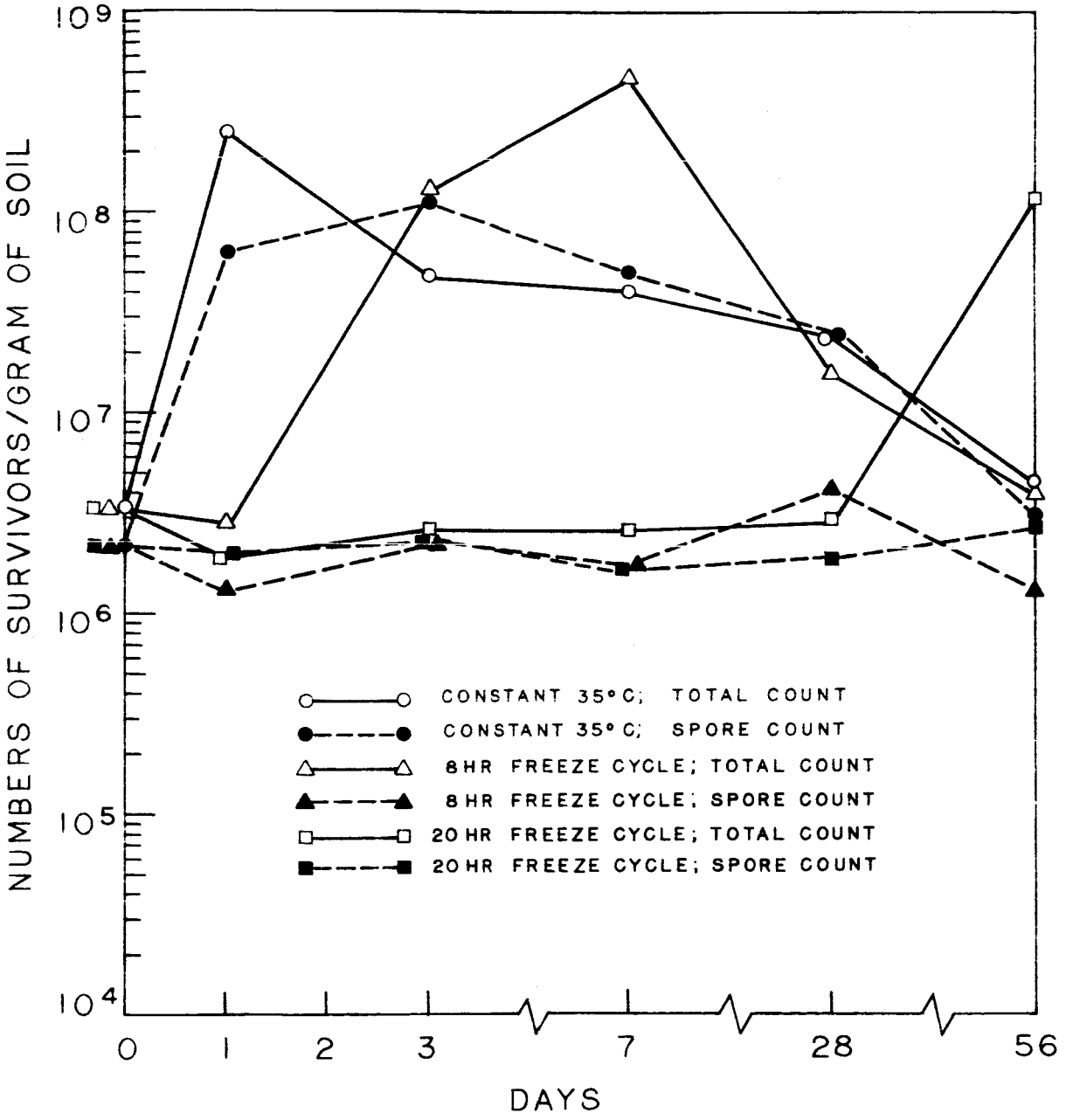

FIG. 4 THE EFFECT OF EARTH ATMOSPHERE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

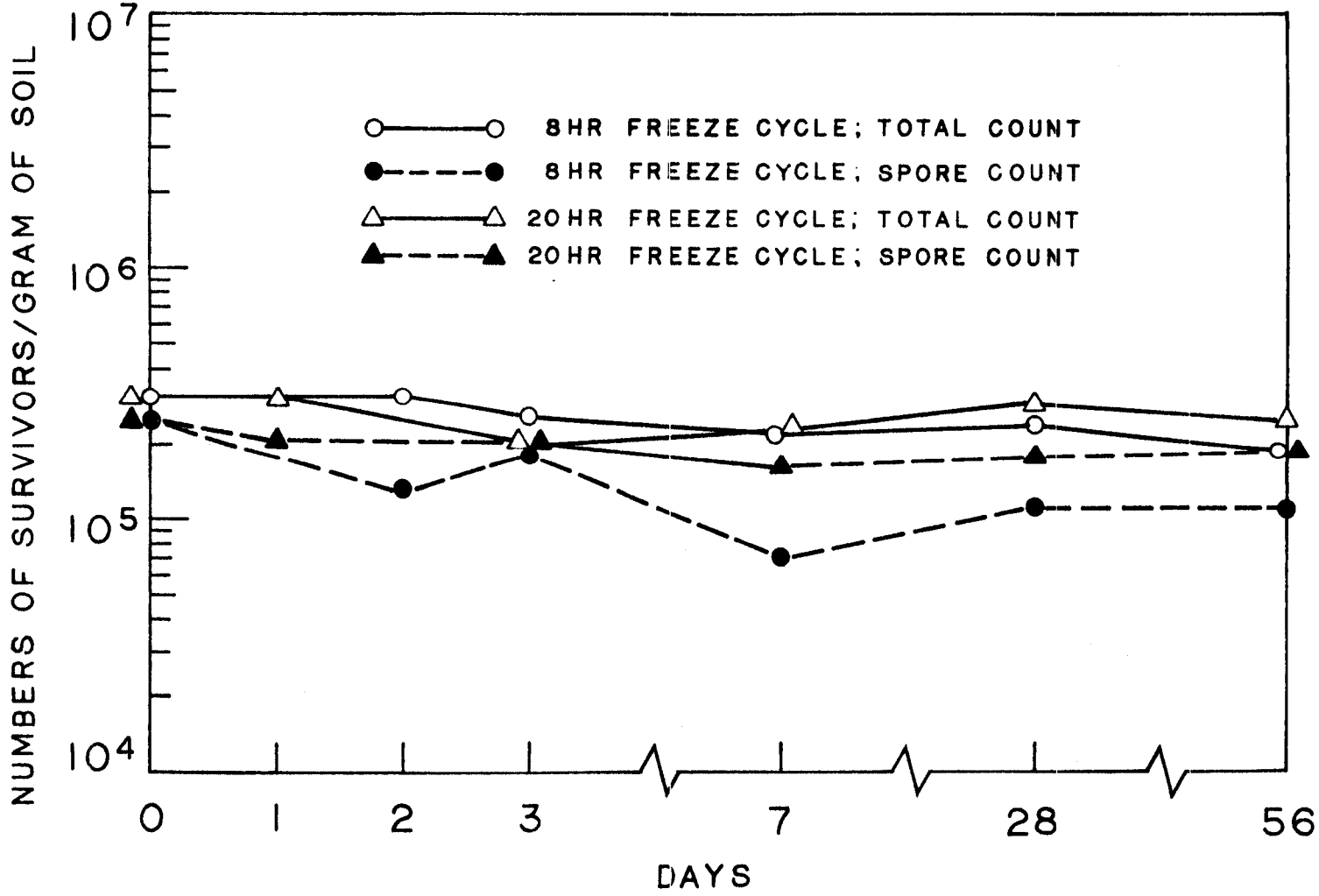

FIG. 5 THE EFFECT OF 100% CARBON DIOXIDE AT 10mb PRESSURE ON BACILLUS CEREUS SPORES.

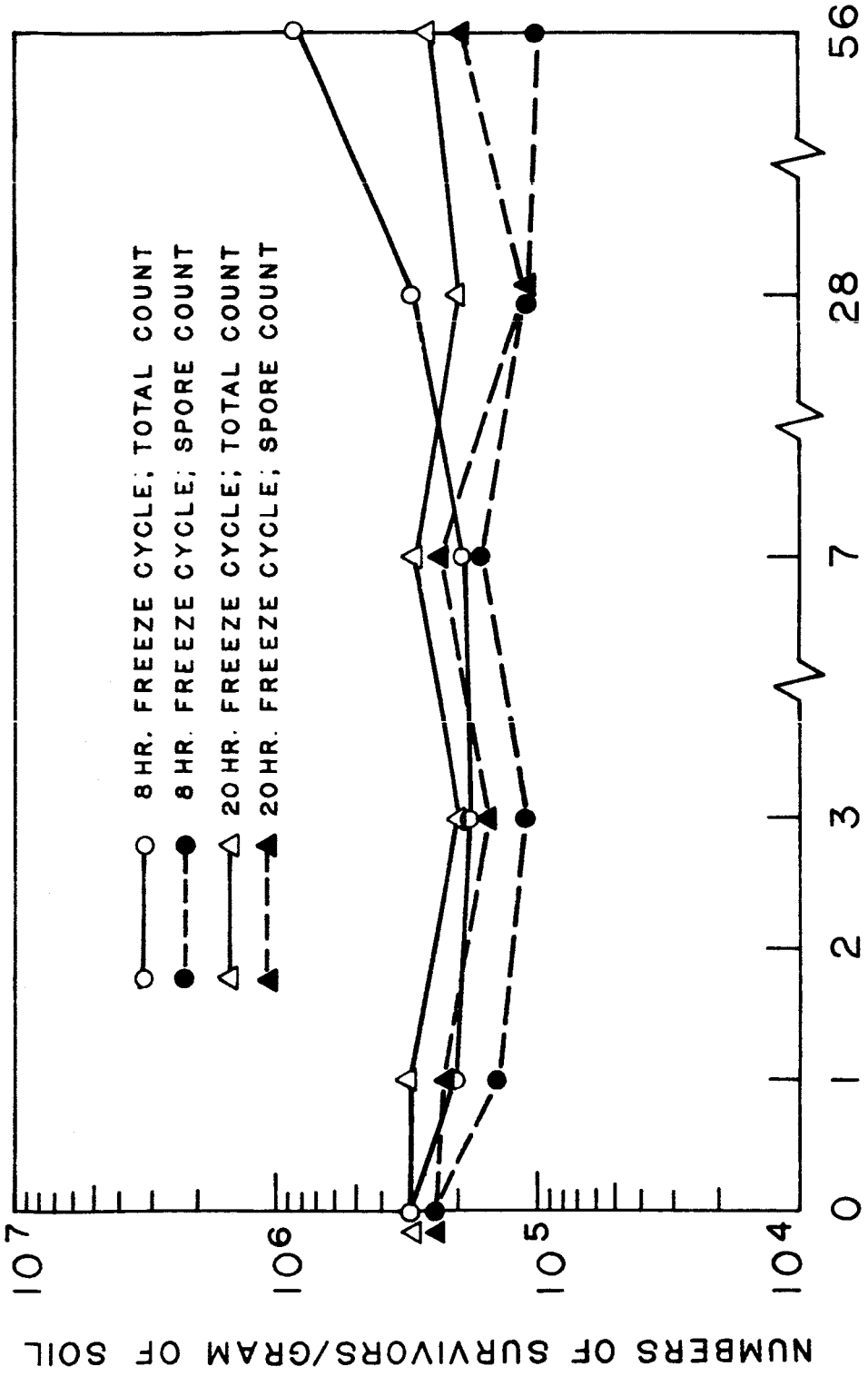

FIG. 6 THE EFFECT OF 67% CARBON DIOXIDE AT 25mb PRESSURE ON BACILLUS CEREUS SPORES.

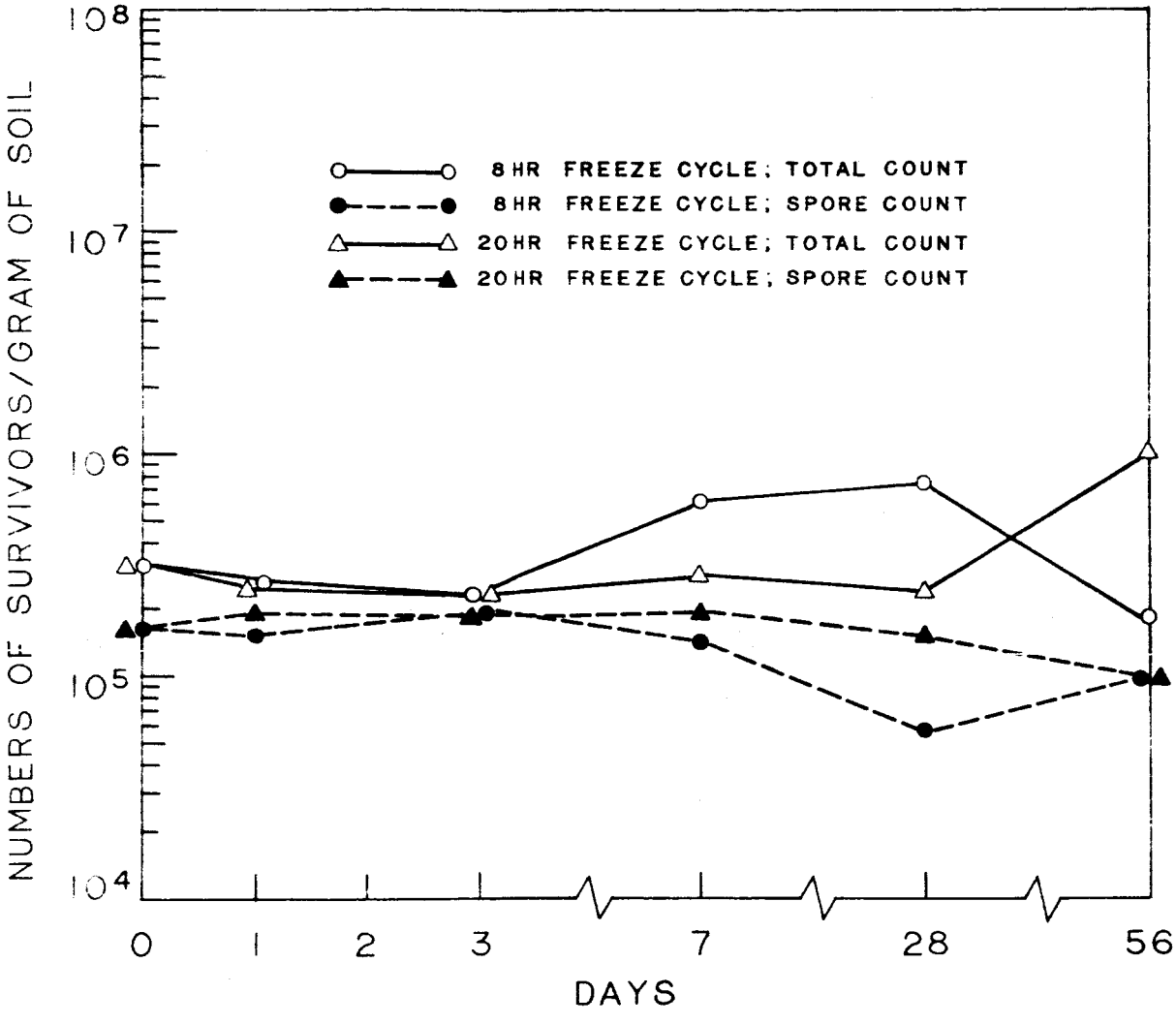

FIG. 7 THE EFFECT OF 37% CARBON DIOXIDE AT 40mb PRESSURE ON BACILLUS CEREUS SPORES.

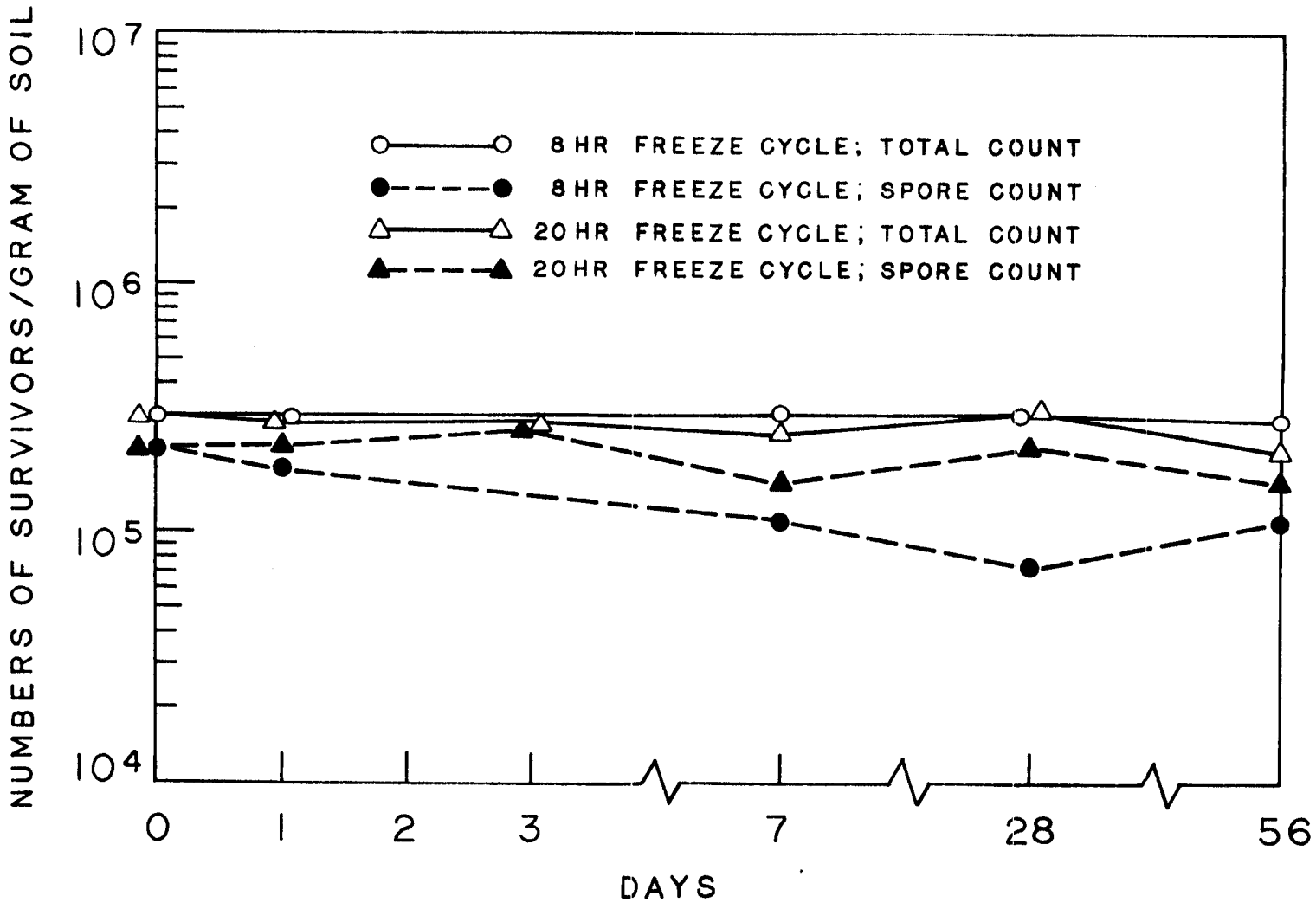

FIG. 8 THE EFFECT OF 37% CARBON DIOXIDE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

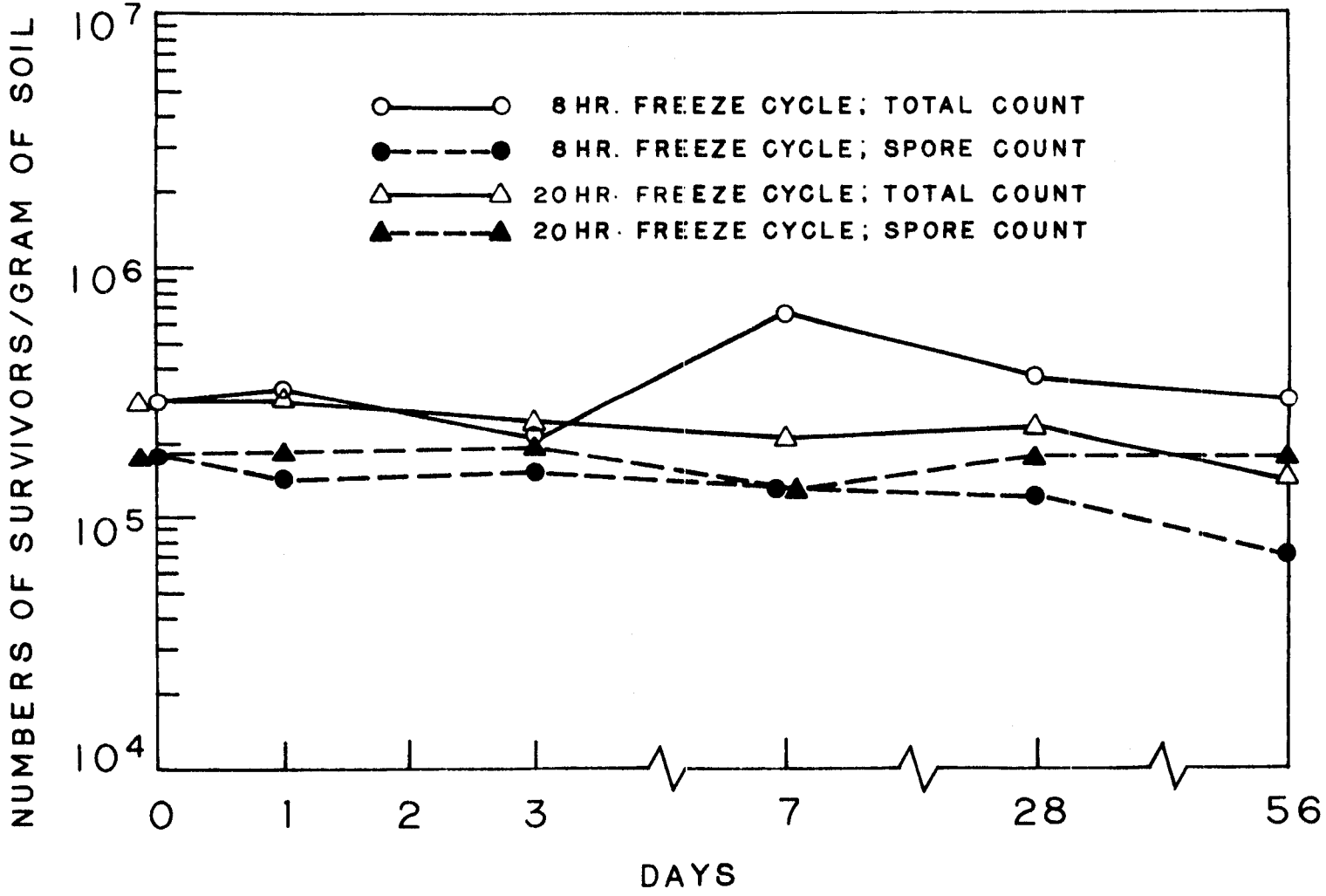

FIG. 9 THE EFFECT OF 67% CARBON DIOXIDE AT 98 mb PRESSURE ON BACILLUS CEREUS SPORES.

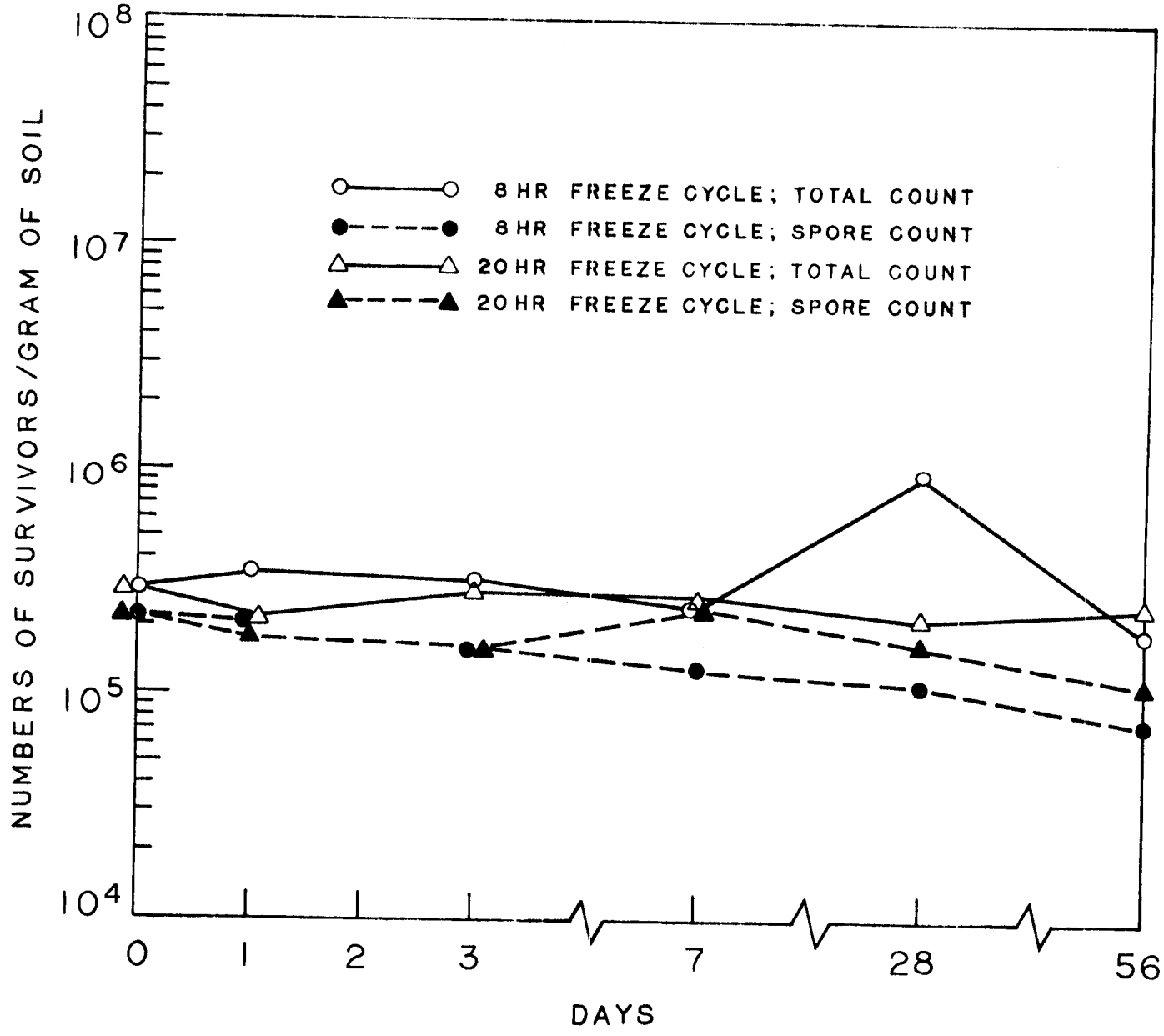

FIG. 10 THE EFFECT OF 100% CARBON DIOXIDE AT 98mb PRESSURE ON BACILLUS CEREUS SPORES.

2. S. aureus

Studies to determine the effects of barometric pressure and carbon dioxide concentrations on the growth response of S. aureus were initiated. Figure 11 shows the effect of 40 mb pressure Earth atmosphere with 8 and 20 hr. diurnal freeze cycles on S. aureus. A 10% moisture was used. The growth response of this organism was more rapid than that observed with any organism studied thus far. S. aureus grew well in environments with both 8 and 20 hr. freeze cycles.

B. Soil Ecology Studies

After 56 days, tests were concluded on the growth response of B. cereus, P. aeruginosa, PA 3679, and S. aureus in an alkaline desert soil at 99% relative humidity with Earth atmosphere at 1013 mb. The tubes inoculated with PA 3679 contained 5% carbon dioxide and 95% nitrogen at 1013 mb pressure. Growth was determined for both constant 35°C and 8 hr. diurnal freeze cycle. The tests with L. plantarum were carried out only to seven days.

Table 1 shows the results obtained. S. aureus was not affected by the inoculation procedures, and approximately 100% recovery was obtained. Only 1% of the L. plantarum and none of the P. aeruginosa cells were recovered. The poor recovery of these latter two organisms was attributed to

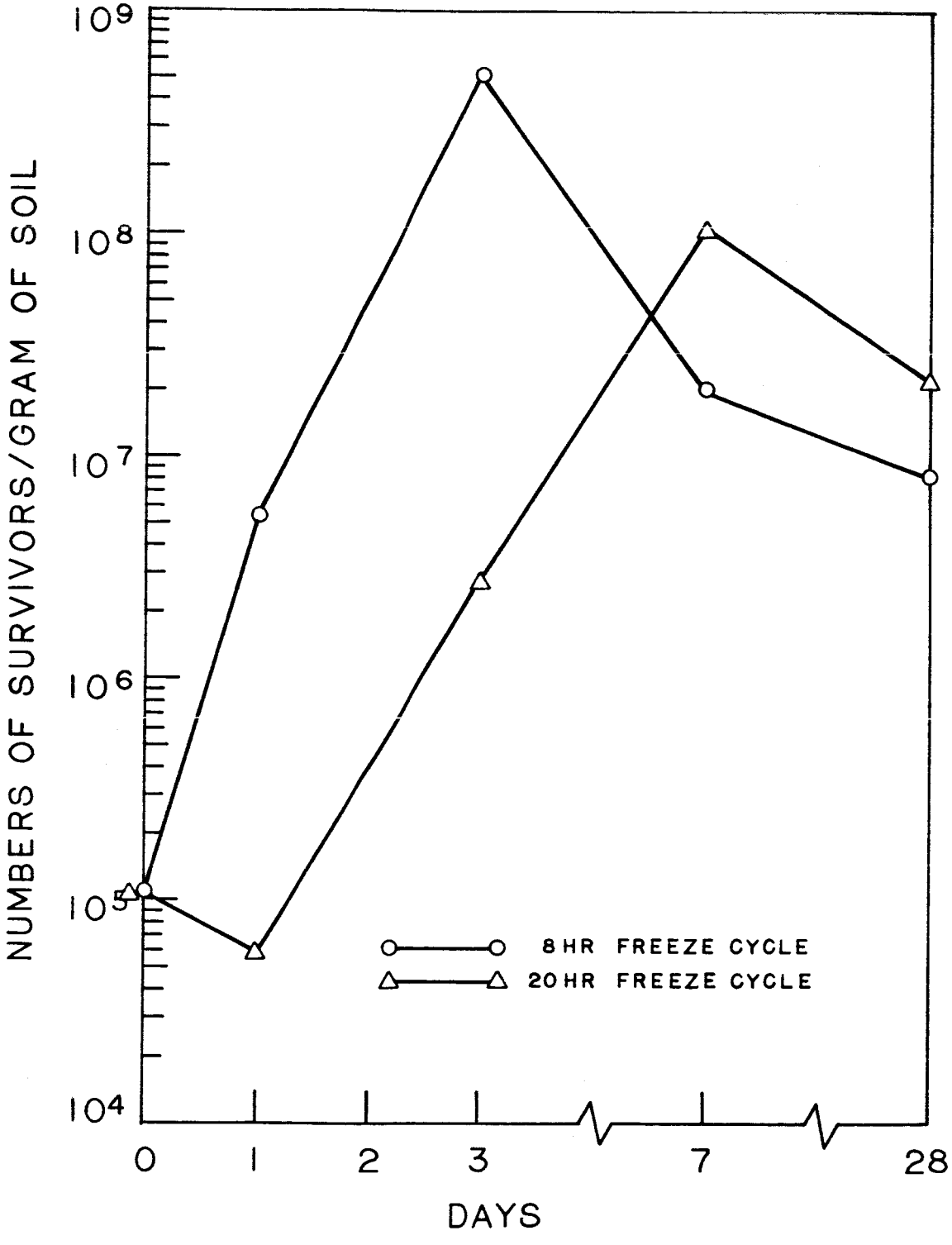

FIG. II THE EFFECT OF EARTH ATMOSPHERE AT 40mb PRESSURE ON STAPHYLOCOCCUS AUREUS.

Table 1

SURVIVAL OF SELECTED BACTERIA IN A CALIFORNIA DESERT SOIL

Day	Inoculum, cells/g of soil	Number of Organisms Recovered/g of Soil: *Lactobacillus plantarum* Constant[a]	*Lactobacillus plantarum* Diurnal[b]	*Pseudomonas aeruginosa* Constant[a]	*Pseudomonas aeruginosa* Diurnal[b]	*Staphylococcus aureus* Constant[a]	*Staphylococcus aureus* Diurnal[b]
0	10^5	2.3×10^3	2.3×10^3	<100	<100	1.7×10^5	1.7×10^5
	10^4	300	300	<50	<50	1.4×10^4	1.4×10^4
	10^3	<50	<50	<50	<50	4.5×10^3	4.5×10^3
7	10^5	<50	<50	<50	<50	100	200
	10^4	<50	<50	<50	<50	<50	100
	10^3	<50	<50	<50	<50	<50	<50
28	10^5			<50	<50	125	<50
	10^4			<50	<50	<50	<50
	10^3			<50	<50	<50	<50
56	10^5			<50	<50	<50	<50
	10^4			<50	<50	<50	<50
	10^3			<50	<50	<50	<50

a - Incubation at constant 35°C.

b - Incubation with diurnal freeze (8 hrs.) thaw cycle (-65 to 30°C).

the pH of the soil, which was 8.0. The alkalinity of the soil could also be responsible for the rapid decay of S. aureus during the experiment. No viable S. aureus cells were recovered from tubes inoculated with 10^3, 10^4, and 10^5 cells after 7, 28, and 56 days, respectively.

Figures 12 and 13 show the effect of the same environment on the survival of B. cereus. B. cereus spores remained viable with less than 1 log reduction at all inoculum levels at both constant 35°C and diurnal freeze-thaw cycle. The slight decrease in total and spore counts over the initial 7 to 28 days indicates some spore germination with subsequent death. However, the plateauing of the total and spore counts over the final 28 day period was caused by the spores not germinating but remaining viable. Significant vegetative growth of B. cereus did not occur.

The behavior of PA 3679 spores in the desert soil in an atmosphere of 5% carbon dioxide and 95% nitrogen at 1013 mb pressure was similar to that of B. cereus (Figures 14 and 15). PA 3679 spores remained viable at all inoculum levels. More fluctuation in total and spore counts occurred with PA 3679 than with B. cereus, and plateauing of total and spore counts did not occur. Although there was a steady decline in viable cells, the decrease was generally less than 1 log cycle when compared with the initial count.

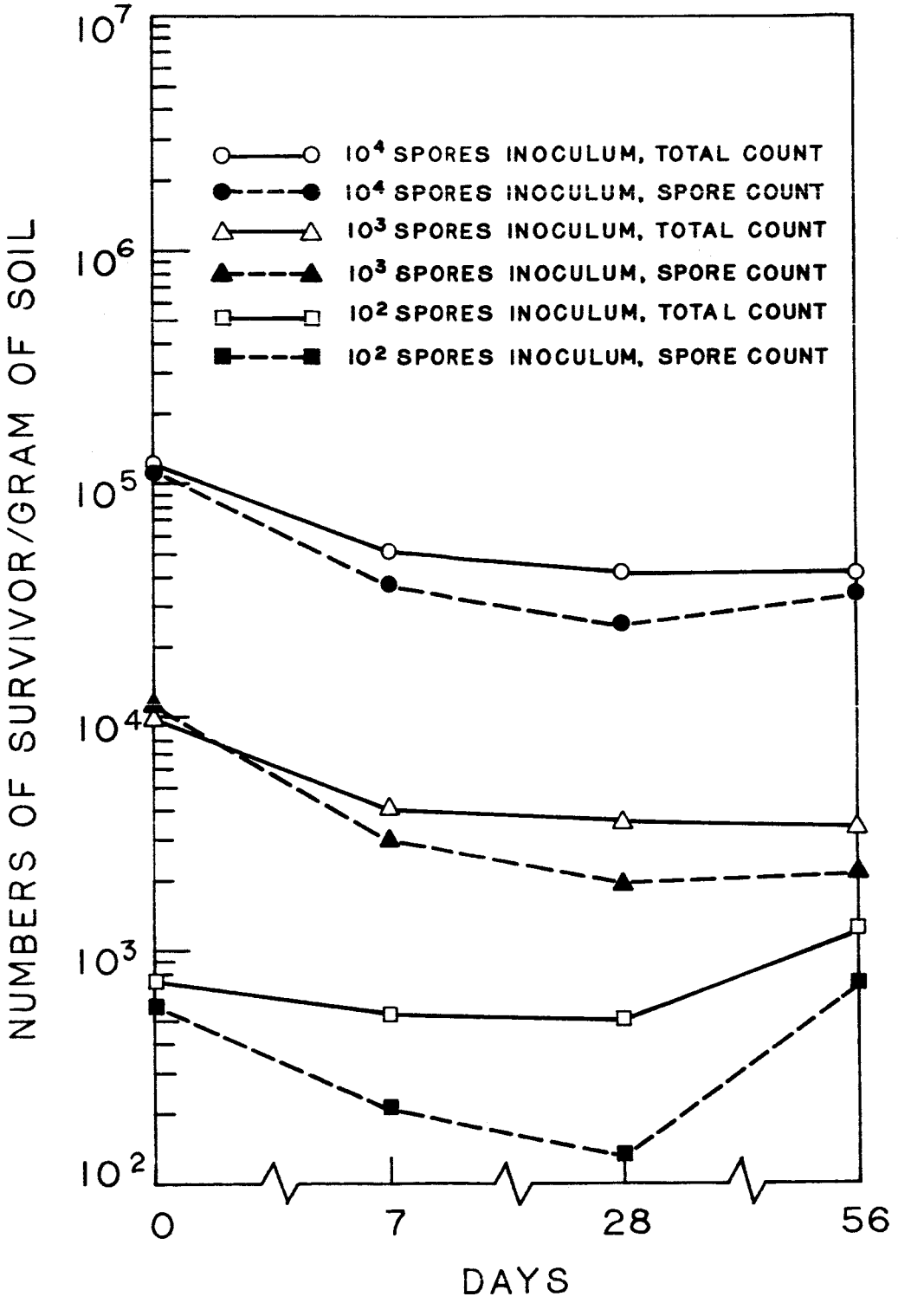

FIG. 12 SURVIVAL OF BACILLUS CEREUS SPORES IN A CALIFORNIA DESERT SOIL AT CONSTANT 35° C.

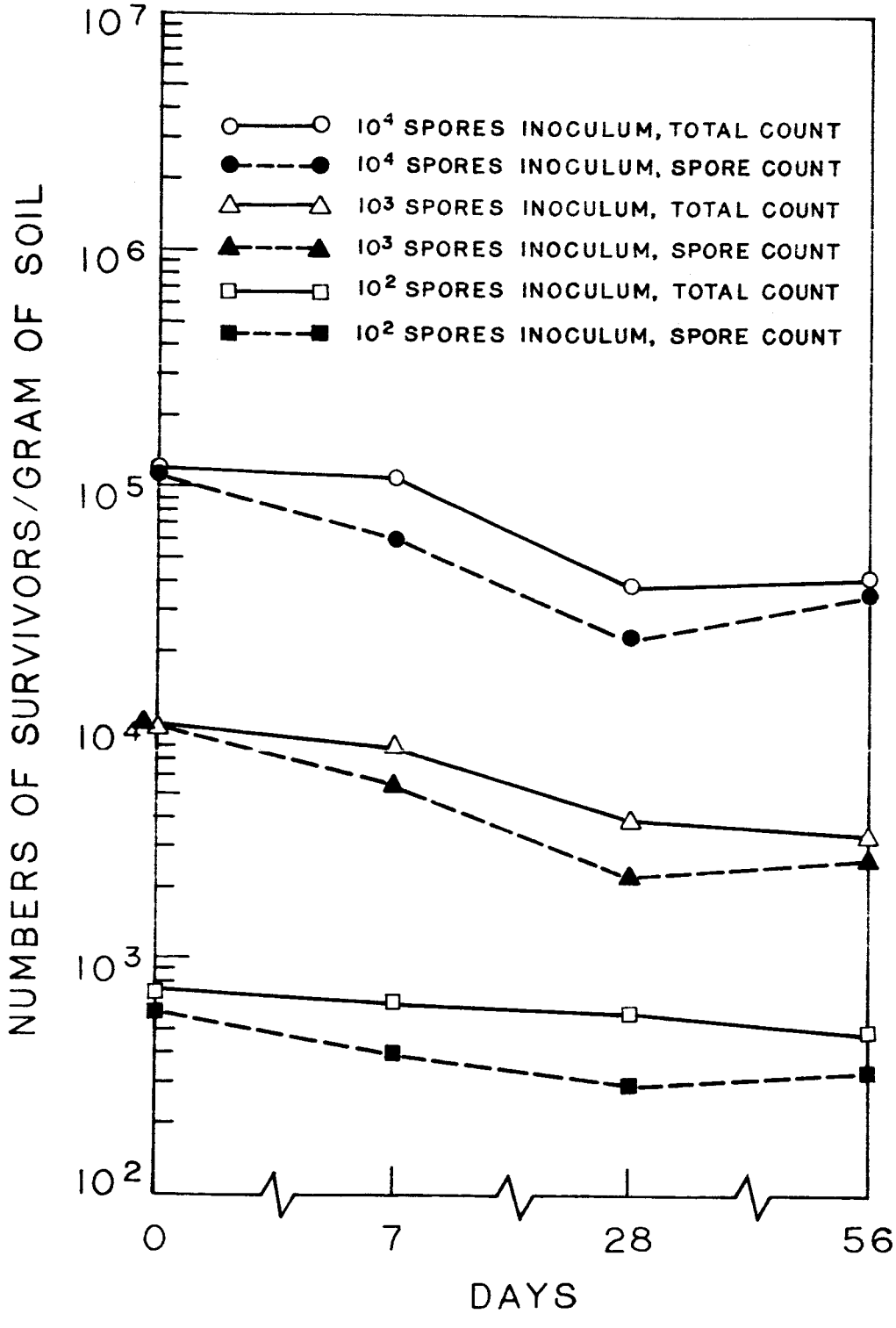

FIG. 13 SURVIVAL OF BACILLUS CEREUS SPORES IN A CALIFORNIA DESERT SOIL WITH A DIURNAL TEMPERATURE CYCLE.

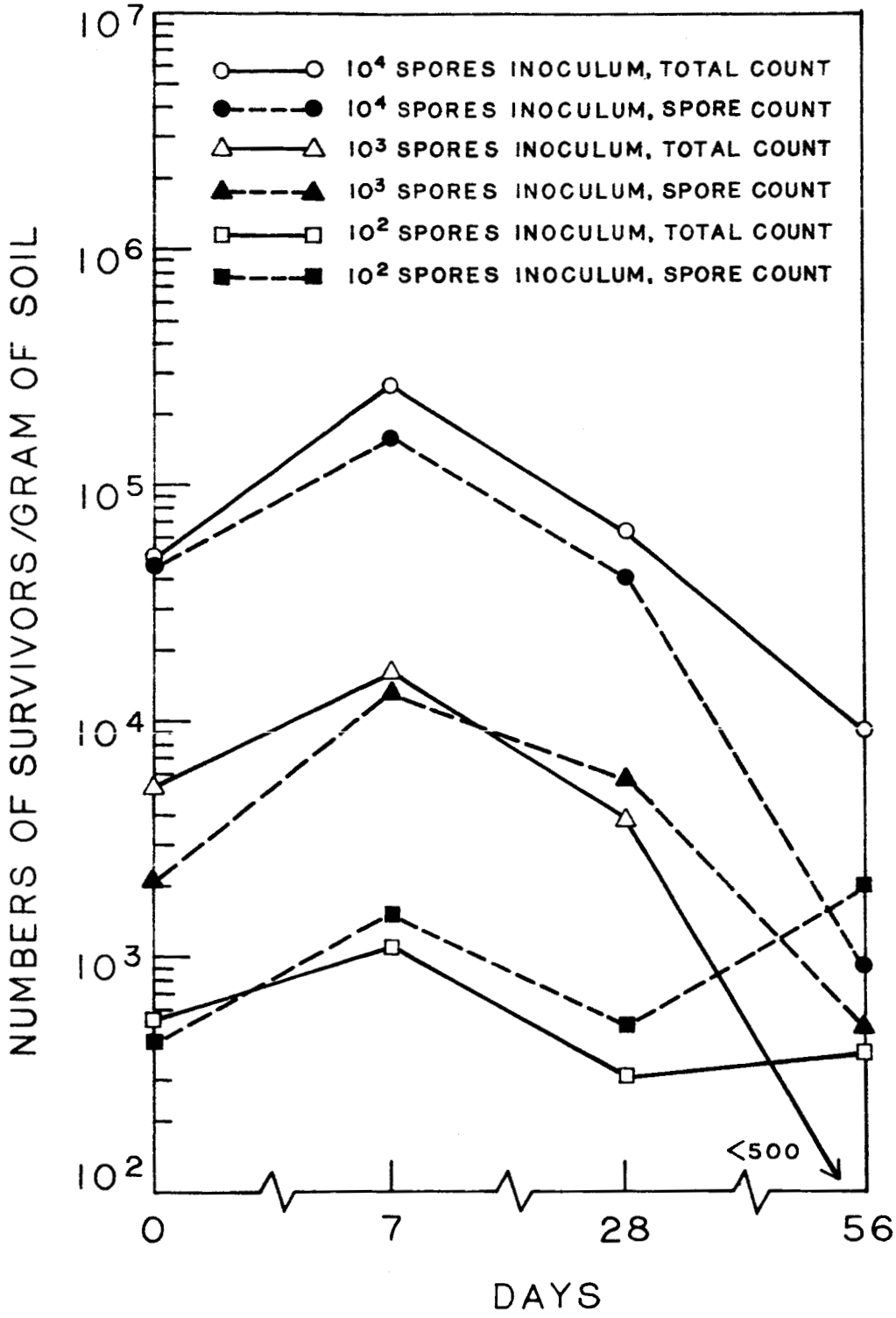

FIG.14 SURVIVAL OF PA 3679 SPORES IN A CALIFORNIA DESERT SOIL AT CONSTANT 35° C.

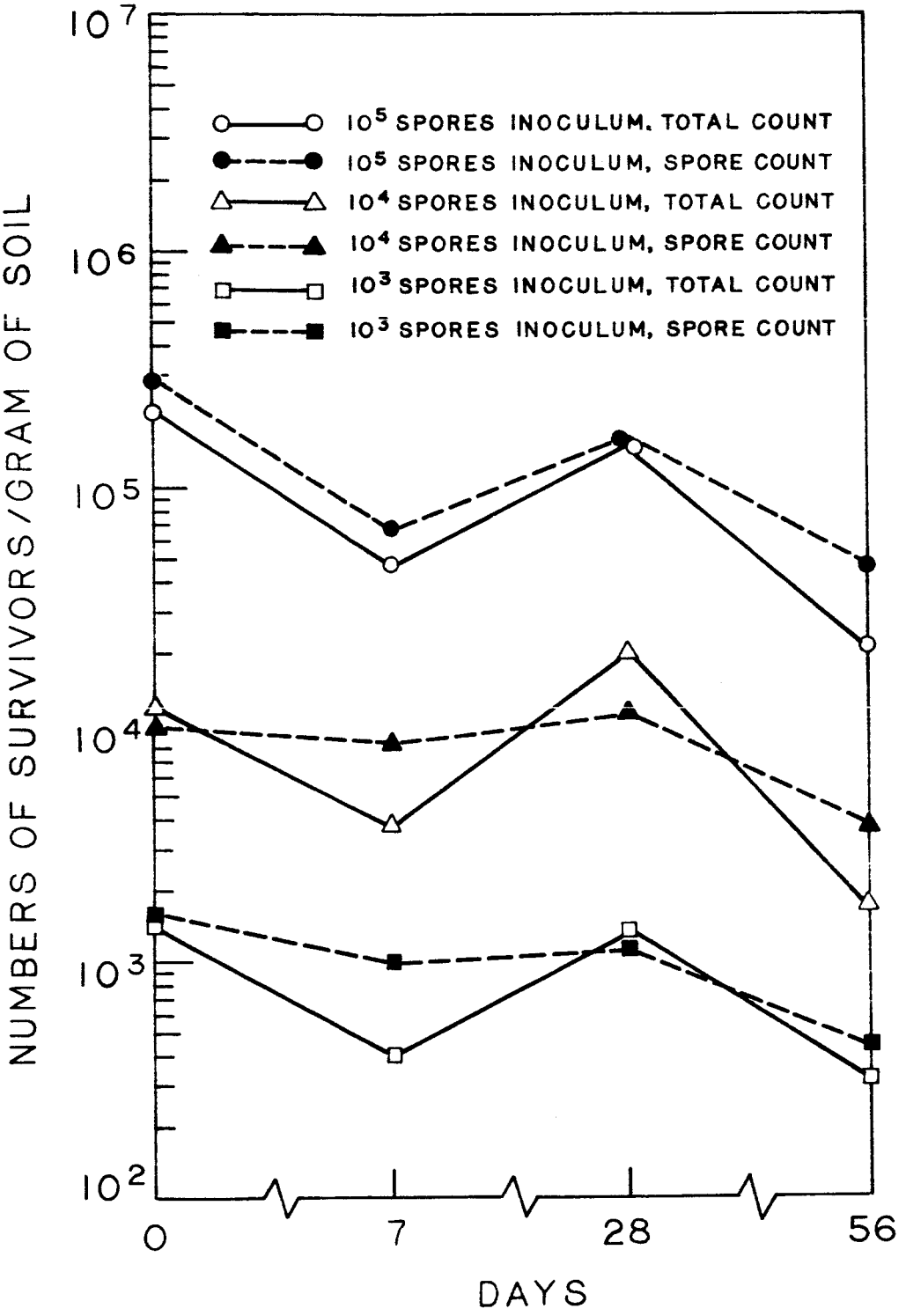

FIG.15 SURVIVAL OF PA 3679 SPORES IN A CALIFORNIA DESERT SOIL WITH A DIURNAL TEMERATURE CYCLE.

In conclusion, the soil ecology studies to date indicate that L. plantarum, P. aeruginosa, and S. aureus do not survive in an alkaline desert soil at initial cell populations as high as 10^5 cells/g of soil. Spores of B. cereus and PA 3679 survive in this environment at initial inoculum as low as 10^2 spores/g of soil with less than a 1 log die-off.

IV. SUMMARY

B. cereus spore germination was not inhibited in air at reduced pressures of 10, 25, and 40 mb, but the growth was less than at 98 mb.

Carbon dioxide concentrations of 37, 67, and 100% at pressures of 40, 25, and 10 mb, respectively, and the same carbon dioxide concentrations at 98 mb pressure inhibited B. cereus spore germination during 8 and 20 hr. diurnal freeze cycles for at least 56 days. The carbon dioxide did not impair cell viability.

The growth response of S. aureus was very rapid with both the 8 and 20 hr. diurnal freeze cycles.

L. plantarum, P. aeruginosa, and S. aureus did not survive in desert soil even at an initial cell population of 10^5 cells/g of soil. Spores of B. cereus and PA 3679 did survive at initial populations as low as 10^2 spores/g of soil.

V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. Bruce T. Anderson, Miss Majorie Ewing, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks, C16678, C16684, C16876, C16882, C16888, C16889, C16938, and C16949.

Respectfully submitted,

IIT RESEARCH INSTITUTE

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Research Bacteriologist
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Approved by:

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Assistant Director
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CAH:hm

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